

mRNA extraction and RT-PCR

Total RNA from 50–150 mg of crushed white adipose tissue was isolated using the technique described by Chomczynski and Sacchi (ref. 19 in main text)). Briefly, samples were dissolved in GSC solution (4 mol/L guanidinium isothiocyanate, 24 mmol/L sodium citrate, 0,5% Sarcosyl) + β -mercaptoethanol (100 mmol/L) and sequentially mixed with sodium acetate pH 4.1, water-saturated phenol and chloroform for extraction. After phase separation RNA was precipitated with isopropanol. cDNA was synthesized from 250 ng of total RNA in a final volume of 20 μ l using 0,1 μ g /rxn random primers (Promega, Dubendorf, Switzerland) and 200U/rxn MMLV reverse transcriptase (Promega) in 50mM Tris-HCl pH 8.3, 75mM KCl, 3mM MgCl₂, 10mM DTT, 312 μ M dNTP at 37° for 1 hour. Quantitative RT-PCR was performed in iQ SYBR Green Supermix BioRad buffer using the primers indicated in the following table:

GENE	Forward primer	Reverse primer
GLUT 4	TGGACCTGTAACCTCATCGTTG GCATGG	CCAAGCAGGAGGACGGCAAATA GAAGG
FABP4	CCTTTGTGGGGACCTGGAAA	TGACCGGATGACGACCAAGT
SREBP1c	TGGTCTTCCTGTGTCTGACCTG CAACC	ACCCGAAGCATCAGAGGGAGTG AGG
FAS	CACAGACGATGACAGGAGGTG GAAGG	TTGGACAGATCCTTCAGCTTCC AGACC
CEBP α	GCACGCGTCTCCCGCGCACTT GG	CCAGGCTGCAGGTGCATGGTGG TCTGG
PPAR γ	GTGATGGATGACCACTCCCAT TCCTTTG	CAGCAACCATTGGGTCAGCTCTT GTG
SCD1	TGGGAAAGTGAAGCGAGCAAC CG	AGAGGGGCACCTTCTTCATCTTC TC
IL-6	TCCTACCCCAACTTCCAATGCT C	TTGGATGGTCTTGGTCCTTAGCC
TNF α	CTCTGACCCCCATTACTCTGAC	CTTCAGCATCTCGTGTGTTTC
CD68	AATGTGTCCTTCCCACAAGC	GGCAGCAAGAGAGATTGGTC
IL-10	GGCTCAGCACTGCTATGTTGC C	AGCATGTGGGTCTGGCTGACTG
G6PDH	CACAGTGGATGACATCCGCAA	GCAGGGCATTGATGTGGCT
CHREBP	GATCCGACACTCACCCGCC	CCCGGCATAGCAACTTGAGG
Cyclophilin	TCAGGGCTCTTGAAGTCCC	CAGAAAATCACAGCAGCCAAC

Samples were incubated in the iCycler instrument (BioRad, iCycler iQ™, Version 3.1.7050) for an initial denaturation at 95° C for 3 min, followed by 40 cycles of amplification. Each cycle consisted of 95° C for 10 sec, 60 or 62° C for 45 sec, and finally 95° C, 55° C and 95° C for 1 min each. Green I fluorescence emission was determined after each cycle. The relative amount of each mRNA was quantified by using the iCycler software. Amplification of specific transcripts was confirmed by melting curve profiles generated at the end of each run. Cyclophilin was used as an invariant control for each study and the relative quantification for a given gene was corrected for the cyclophilin mRNA values.

Supplementary Table 1. Gene markers of inflammation in EWAT and IWAT of control (C) and refed (RF) rats on a low fat (LF) or a high fat (HF) diet on day 3-4 of refeeding

All values are means ± SE (n= 6-8), and refer to quantification for a given gene relative to cyclophilin mRNA values. NS= no significant difference by ANOVA

	C-LF	C-HF	RF-LF	RF-HF	Two-factor ANOVA		
					Group effect	Diet effect	Group*Diet interaction
<u>EWAT</u>							
CD-68	2.51 ± 0.26	2.91 ± 0.38	2.13 ± 0.49	2.83 ± 0.61	NS	NS	NS
TNF α	0.71 ± 0.08	0.75 ± 0.04	0.57 ± 0.10	0.66 ± 0.07	NS	NS	NS
IL-6	0.48 ± 0.07	0.28 ± 0.05	0.27 ± 0.02	0.50 ± 0.17	NS	NS	NS
IL-10	0.81 ± 0.25	0.84 ± 0.22	0.57 ± 0.13	0.77 ± 0.23	NS	NS	NS
<u>IWAT</u>							
CD-68	1.25 ± 0.23	1.00 ± 0.36	0.61 ± 0.21	0.70 ± 0.22	NS	NS	NS
TNF α	1.85 ± 0.64	1.27 ± 0.27	0.53 ± 0.17	1.21 ± 0.54	NS	NS	NS
IL-6	4.15 ± 1.5	6.5 ± 1.6	0.85 ± 0.19	1.84 ± 0.31	$p<0.01$	NS	NS
IL-10	1.38 ± 0.4	2.45 ± 0.57	0.43 ± 0.11	1.55 ± 0.93	NS	$p<0.05$	NS

Supplementary Table 2. Inflammatory markers in plasma (pg/ml) , as well as plasma adiponectin (μg/ml) and leptin (mg/ml), in control (C) and refed (RF) rats on low fat (LF) diet or high fat (HF) diet on day 3-4 of refeeding.

All values are means ± SE (n= 6-8). NS= no significant difference by ANOVA.

	C-LF	C-HF	RF-LF	RF-HF	Two way ANOVA		
					Group effect	Diet effect	Group * Diet interaction
TNFα	523 ± 75	446 ± 24	416 ± 37	391 ± 32	NS	NS	NS
IL-6	614 ± 117	554 ± 61	652 ± 63	687 ± 103	NS	NS	NS
IL-10	592 ± 26	584 ± 12	609 ± 47	612 ± 29	NS	NS	NS
IFNγ	768 ± 59	642 ± 47	671 ± 41	693 ± 20	NS	NS	NS
IL-1 beta	36 ± 1	35 ± 1	37 ± 1	38 ± 1	NS	NS	NS
IL-1 RA	66 ± 2	59 ± 7	64 ± 1	70 ± 2	NS	NS	NS
IL-1 alpha	49 ± 1	47 ± 1	47 ± 2	50 ± 1	NS	NS	NS
CRP	50 ± 3	47 ± 2	51 ± 2	53 ± 6	NS	NS	NS
Adiponectin	2.31 ± 0.12	2.24 ± 0.09	2.13 ± 0.06	2.12 ± 0.12	NS	NS	NS
Leptin	2.13 ± 0.31	2.24 ± 0.16	2.48 ± 0.31	2.50 ± 0.27	NS	NS	NS

Supplementary Table 3. Data on tissues harvested on day 3-4 of refeeding in refed (RF) and control (C) groups on low fat (LF) or high fat (HF) diet

Hepatic lipids (mg lipids/g tissue)

	C-LF	C-HF	RF-LF	RF-HF	Two way ANOVA		
					Group effect	Diet effect	Group * Diet interaction
	72.2 ± 4.8	87.4 ± 4.9	89.8 ± 8.0	80.9 ± 4.7	NS	NS	NS

Aconitase activity (U/mg protein)

	C-LF	C-HF	RF-LF	RF-HF	Two way ANOVA		
					Group effect	Diet effect	Group * Diet interaction
EWAT	518 ± 74	513 ± 74	508 ± 75	419 ± 24	NS	NS	NS
IWAT	389 ± 19	358 ± 61	445 ± 51	308 ± 104	NS	NS	NS
Liver	259 ± 27	211 ± 20	218 ± 27	258 ± 27	NS	NS	NS

Superoxide dismutase activity (SOD) (U/mg protein)

	C-LF	C-HF	RF-LF	RF-HF	Two way ANOVA		
					Group effect	Diet effect	Group * Diet interaction
EWAT	27.4 ± 1.1	30.7 ± 0.5	27.1 ± 0.7	27.9 ± 1.1	NS	NS	NS
IWAT	23.5 ± 1.4	24.5 ± 0.8	22.5 ± 0.9	24.6 ± 0.9	NS	NS	NS
Liver	3.59 ± 0.37	3.60 ± 0.30	3.41 ± 0.20	3.33 ± 0.20	NS	NS	NS

Supplementary Figure 1. Dietary fat exacerbates catch-up fat and impairs glucose tolerance

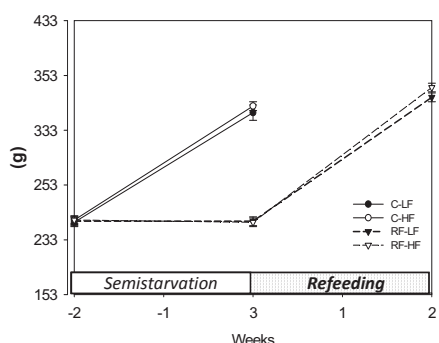
Panel A and B: Body weight and body fat changes in response to 2 wk of semistarvation and isocaloric refeeding in refeed (RF) rats and control (C) animals on a low-fat (LF) or high-fat (HF) diet. (Body composition determination as described in ref. 6 in main text)

Panel C and D: Glucose tolerance test (GTT). Plasma glucose and insulin before and over 2h after i.p. glucose load (2g/kg bw), as previously described (ref. 7 in main text)

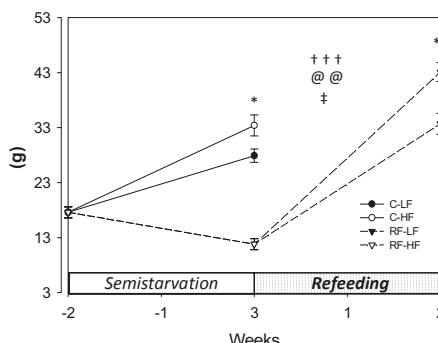
Panel E and F: Incremental plasma glucose and insulin area-under-the-curve (AUC) during GTT.

Statistics: All values are means \pm SE (n = 6-8). Statistical significance of differences, assessed by 2-factor ANOVA, is indicated as follows: † denotes Group effect (RF vs C), @ denotes Diet effect (LF vs HF) and ‡ denotes Group x Diet interaction. The symbol * denotes significant difference by post-hoc pairwise comparison between diets within either refeed animals (RF-LF vs RF-HF) or within control animals (C-LF vs C-HF). Single, double and triple symbols imply $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. Note that for plasma glucose GTT, pairwise differences are observed between RF-HF vs RF-LF but not between C-HF and C-LF.

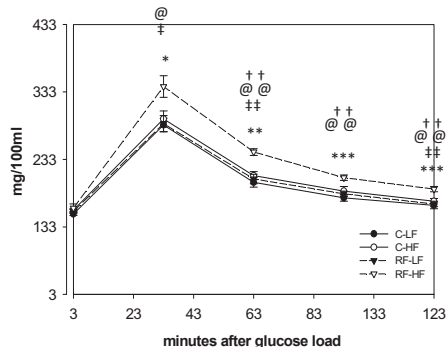
A Body Weight



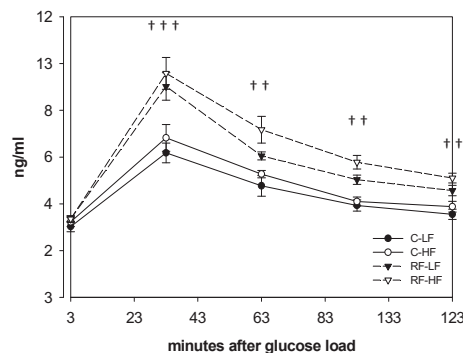
B Body Fat



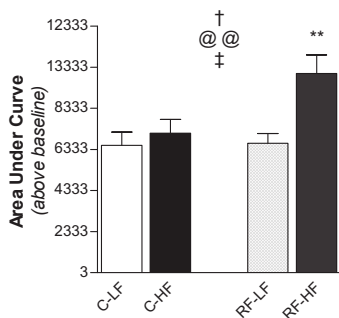
C Plasma glucose GTT



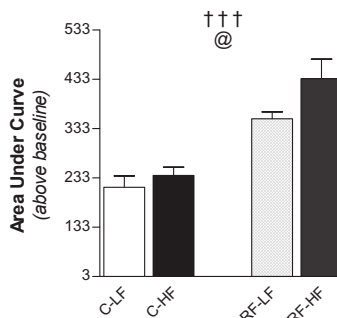
D Plasma insulin GTT



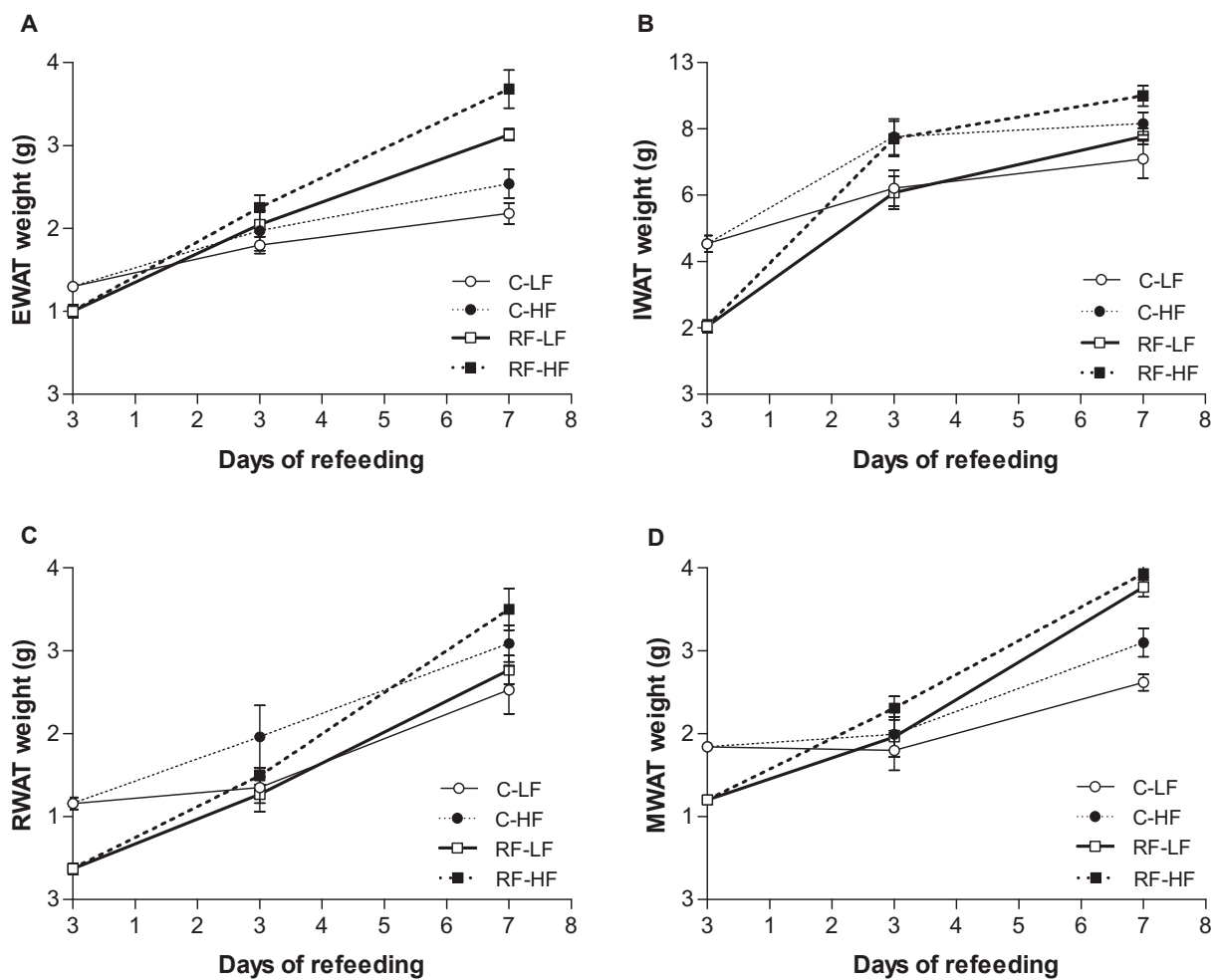
E Plasma glucose AUC



F Plasma insulin AUC



Supplementary Figure 2. Kinetics of adipose tissue recovery during first week of catch-up fat
Weight of epididymal fat (EWAT), inguinal fat (IWAT), retroperitoneal (RWAT) and mesenteric fat (MWAT) during the first week of refeeding after semistarvation. Note that on day 3-4 of refeeding, the weight of adipose tissues from the refed (RF) animals are not yet higher than that of their respective diet controls (C).



Supplementary Figure 3. *de-novo* lipogenesis (DNL), assessed as rate of incorporation of ^3H and ^{14}C in total lipids from adipose tissues (EWAT, IWAT) and liver of refeed (RF) and control (C) rats on day 3-4 of refeeding on a low fat (LF) or high fat (HF) diet. All values are means \pm SE (n=6-8). Statistical significance of differences, assessed by 2-factor ANOVA, is indicated as follows: † denotes Group effect (RF vs C), @ denotes Diet effect (LF vs HF) and ‡ denotes Group x Diet interaction. The symbol * denotes significant difference by post-hoc pairwise comparison between diets within either refeed animals (RF-LF vs RF-HF) or within control animals (C-LF vs C-HF). Single, double and triple symbols imply $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively

